

UNIVERSITI TEKNOLOGI MARA

**QUANTITATIVE DETERMINATION ON
BIODEGRADATION OF PAHs BY BACTERIA
FROM OIL SLUDGE**

NORHAFIZA BINTI ABDUL GHANI

Dissertation submitted in partial fulfillment of the requirements
for the degree of

Master of Science in Civil Engineering (Environmental)

Faculty of Civil Engineering

November 2008

ABSTRACT

The biodegradability of petroleum waste product was evaluated using oil sludge taken from Exxon Mobil petroleum refinery treatment plant in Kerteh, Terengganu. This study was a further extension of studies conducted by Dzulkafli (2007) where the qualitative analysis was established. The analysis involves the utilization of bacterial strains most effective in PAHs degradation as classified by Dzulkafli (2007). The PAHs adopted were phenanthrene, anthracene and dibenzothiophene which were used as source of carbon. PCR and DNA Sequencing were conducted to confirm the identity of bacterial strain classified by Dzulkafli (2007). These molecular techniques provides up to 90% accuracy of identification. Using these techniques these three strains identified belong to genus of *Pseudomonas* namely; Uncultured *Pseudomonas* sp. clone 2-A, *Pseudomonas Stutzeri* strain HS-D36 and *Pseudomonas Stutzeri* strain LS401. Turbidity test was conducted for growth rates of each strain. *Pseudomonas Stutzeri* strain HS-D36 indicated the highest growth rates followed by Uncultured *Pseudomonas* sp. clone 2-A and *Pseudomonas Stutzeri* strain LS401. Identification of the most effective PAH degrading by bacterial strain was established by determining of average degradation rates. Anthracene proved to be the most degraded by these strains with the highest average degradation rate followed by phenanthrene and dibenzothiophene. While, *Pseudomonas Stutzeri* strain HS-D36 revealed to be the best strain in degrading PAHs, with the highest degradation followed by Uncultured *Pseudomonas* sp. clone 2-A and *Pseudomonas Stutzeri* strain LS401.

Keywords: bacterial strain, biodegradation, DNA Sequencing, oil sludge, PCR, poly aromatic hydrocarbons (PAHs), *Pseudomonas*,

Candidate's Declaration

I declare that the work in this dissertation was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This topic has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

In the event my thesis be found to violate the conditions mentioned above, I voluntarily waive the right of conferment of my degree and agree be subjected to the disciplinary rules and regulations of Universiti Teknologi MARA.

Name of Candidate	NORHAFIZA BINTI ABDUL GHANI
Candidate's ID No.	2007254546
Programme	MASTER SCIENCE CIVIL ENGINEERING (ENVIRONMENTAL) (EC 774)
Faculty	FACULTY OF CIVIL ENGINEERING
Thesis Title	QUANTITTATIVE DETERMINATION ON BIODEGRADATION OF HYDROCARBON BY MICROBE FROM OIL SLUDGE

Signature of Candidate _____

Date _____

TABLE OF CONTENTS

	Page
Title page	
Abstract	i
Candidate's Declaration	ii
Acknowledgements	iii
Table of Contents	iv
List of Tables	viii
List of Figures	ix
List of Abbreviations	xi

CHAPTER 1 INTRODUCTION

1.1	Background of the Study	1
1.2	Problem Statement	3
1.3	Objectives of the Study	4
1.4	Scope and Limitations of Work	4
1.5	Significance of Study	6

CHAPTER 2 LITERATURE REVIEW

2.0	Introduction	7
2.1	Problems of hydrocarbon contaminated soil	7
2.2	Remediation technologies for contaminated soils	8
2.2.1	Soil Vapor Extraction (SVE)	8
2.2.2	Solidification & Stabilization	8
2.2.3	Phytoremediation	9
2.3	Bioremediation	9
2.3.1	Principles of Bioremediation	9
2.3.2	Factors affecting bioremediation of Petroleum Hydrocarbon	11

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Large quantities of organic and inorganic compounds are released into the environment every year as a result of human activities. Contaminated land is a typical consequence of industrial activities such as the petroleum industry. A variety of waste is generated during the petroleum-refining process which requires treatment before they can be safely disposed off (Marin *et al.*, 2005). Oil refineries generate huge volumes of sludge during the refining of crude oil. These sludges have a high content of petroleum-derived hydrocarbons consisting of mainly alkanes and paraffin of 1-40 carbon atoms, along with cycloalkanes and aromatic compounds (Overcash and Pal, 1979). Polycyclic aromatic hydrocarbons (PAHs) are pollutants that can easily enter the environment as a result from the oil spills which can pollute soil and water (Moretto *et al.*, 2005). Indiscriminate and improper disposal and handling of oily sludge contaminates soil which may pose a serious risk to human health as a result of their toxic, mutagenic and carcinogenic properties (Kastner *et al.*, 1994).

There are modern treatment methods that are applicable to the petroleum industry in Malaysia, namely, soil vapor extraction (SVE), natural attenuation, enhanced attenuation, containment and bioremediation (Yin *et al.*, 2007). The selection of remediation technology for clean-up of contaminated land is reliant on various factors such as local soil conditions, hydro geological conditions and the type of contaminants. However, cost plays a major factor for industries in deciding the method to be used for cleaning up contaminated sites.